be associated with significant morbidity. This may reflect our current limitations in staging lung cancer, particularly in detecting early or occult metastases. Several recent studies have demonstrated the ability to detect circulating lung tumour cells by RT-PCR using several gene products and the prognositc value of this investigation. There have been few studies exploring the use of IHC to detect circulating tumour cells. In these studies, IHC was found to be inferior to RT-PCR. The aim of this study was to assess the utility of a novel cell separation method called RosetteSep to generate a concentrated pellet of non-hematologic cells from peripheral blood, which would allow for potentially improved detection of circulating tumour cells by cellular morphology and IHC stains for CEA, CK19, TTF1 and Moc1 compared with RT-PCR using mRNA products of the same genes.

Methods: Forty Stage III or IV, previously untreated, NSCLC patients were accrued at consultation at the Vancouver Cancer Centre (VCC). Twenty healthy volunteer subjects with no known prior malignancy, lung disease or ongoing infection were recruited by letter and poster and accrued at VCC. Each consented subject had a total of 17 ml of peripheral blood drawn. The first 2 ml were discarded to avoid epithelial cell contamination from the needle prick through the skin and the remaining 15 ml were collected into heparin tubes. CD45 positive cells were depleted using samples incubated with RosetteSep CD45 Depletion Cocktail (StemCell Technologies Inc) to create cell concentrate pellet samples. Half of this sample was used to create a cell block and slices were taken for celular morphology and IHC using stains for CEA, CK19, TTF1 and Moc1. The second half of this sample was analyzed using RT-PCR to detect the gene products of CEA, CK19, TTF1 and Moc1.

Results: IHC was reported positive if there was at least 30% cytoplasmic staining for CEA, CK19 and Moc1 and at least 30% nuclear staining for TTF1. Cell morphology was used to confirm the presence of malignant cells in the cell block specimen. The results of RT-PCR were reported as a relative quantity of gene expression in each sample. The detection rate of circulating tumour cells in NSCLC patients by RT-PCR was comparable to the detection rates demonstrated in previous studies. The detection rate of circulating tumour cells by IHC was lower than with RT-PCR but the difference was not significant. No false positives were noted in the healthy volunteers.

Conclusions: This novel cell concentration method improved IHC detection of circulating tumour cells from peripheral blood samples of NSCLC patients. Further refinement of this technique may prove that a smaller volume of blood is required for IHC detection of circulating tumour cells, allowing for better clinical applicability of this investigation. This may ultimately improve "staging" of patients to improve selection of patients for appropriate therapies. Finally, the creation of a cell block would allow for tissue banking for future IHC studies in NSCLC.

D6-07 **Molecular Biology & Prognostic Factors, Thu, 12:30 - 14:15**

A seventy two gene signature and survival in completely-resected non-small-cell lung cancer (NSCLC)

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Background: Current staging methods are imprecise for predicting the outcome of treatment of NSCLC. The goal of this project was to develop a gene expression profile for stage I and II NSCLC that is able to identify patients that have a high risk of disease progression within 2-3 years after initial diagnosis.

Methods: We used whole-genome gene expression microarrays to analyze frozen-tumor samples from 172 patients (pT1&2, N0&1, MO), who had undergone complete surgical resection in 5 European institutions. Randomly generated numbers were used to assign 2/3 of the samples to an algorithm training group with the remaining 1/3 set aside for independent validation. Cox proportional hazards models were used to evaluate the association between the level of expression and patient survival. We used risk scores and nearest centroid analysis to develop a gene-expression model for the prediction of treatment outcome. 10-fold cross validation was used to prevent model over-training.

Results: 72 genes that correlated with survival were identified by analyzing microarray data and risk scores. Based on the expression of these genes, patients in training and validation groups were classified as either high or low risk. Analysis of predicted risk groups revealed significantly different survival distributions for patients in both the training set ($p<0.001$) and independent validation set ($p=0.01$). Genes in our prognostic signature are enriched for genes associated with immune response, antigen binding and protein modification/ubiquination.

Conclusions: Our 72-gene signature is closely associated with overall survival of completely-resected NSCLC patients.